SUPPLEMENTARY DATA

Supplementary Results

Genetic testing

Sequencing of all exons in *HNF1A*, its promoter sequences and key intronic sequences flanking all exons revealed no any additional variants apart from p.A251T.

Copy number variant analysis, undertaken by MLPA and next generation sequencing demonstrated no variation. Using off-target reads from next generation sequencing data to map homozygosity (https://github.com/rdemolgen/SavvySuite/), a 12.2 megabase homozygous region in chromosome 12 encompassing the *HNF1A* gene was confirmed.

In silico characterisation of the p.A251T variant

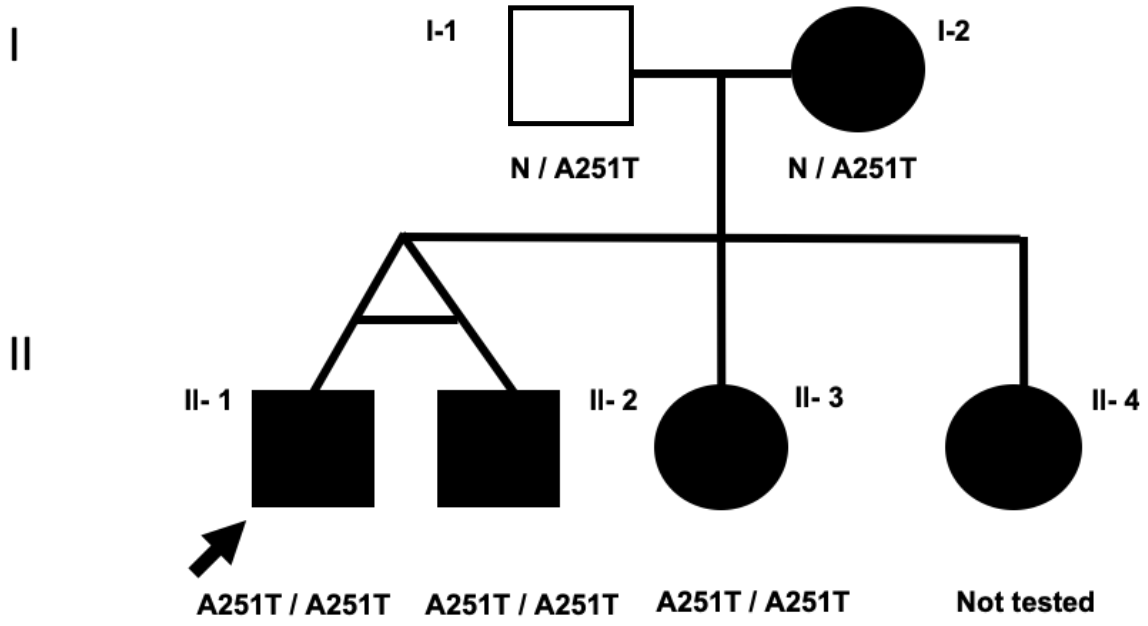
The p.A251T variant is located in a region of *HNF1A* which encodes for a highly conserved POU_H DNA-binding domain. The variant was not present in the GnomAD database (examined July 2019) nor have any other rare variants at this nucleotide position been reported (see supplementary table 2). Residue p.A251 and its flanking residues are strongly conserved in vertebrates (10 out of 10 mammalian orthologs studied). SIFT, PolyPhen-2 and AlignGVGD all predict the p.A251T variant to be likely pathogenic, however based on the Grantham distance (an indicator of similarity between the substitution and constitutive amino acid), a variant of uncertain significance was predicted. The variant was not predicted to create a cryptic splice site.

The DNA binding domain of HNF-1A is an atypical member of the POU family, comprised of the POU-specific (POU_S) and homeodomain (POU_H) sub-domains, both of which contribute to DNA binding (14). Both p.A251 and the MODY-causing variant, p.V246 lie in the POU_H sub-domain (residues 203-279), where their sidechains form part of a hydrophobic patch at the interface with the POU_S sub-domain (supplementary figure 2A). The p.A251T and p.V246L substitutions introduce bulkier sidechains at this interface such that close interaction with the POU_S sub-domain is likely to be adversely affected (supplementary figure 3). Unlike other POU domains, rigidity of the POU_S-POU_H interface appears to be crucial for HNF-1A structure and function, and Chi *et al*. noted that all substitutions made at the interface were detrimental to activity (14). However, in the case of the p.A251T variant, this effect is likely to be compensated by a gain of binding energy through formation of a novel hydrogen bond, predicted between the threonine sidechain and the backbone carbonyl oxygen atom of residue Q176 in the POU_S sub-domain (supplementary figure 3c), thus providing a possible molecular basis for the milder phenotype associated with this variant.

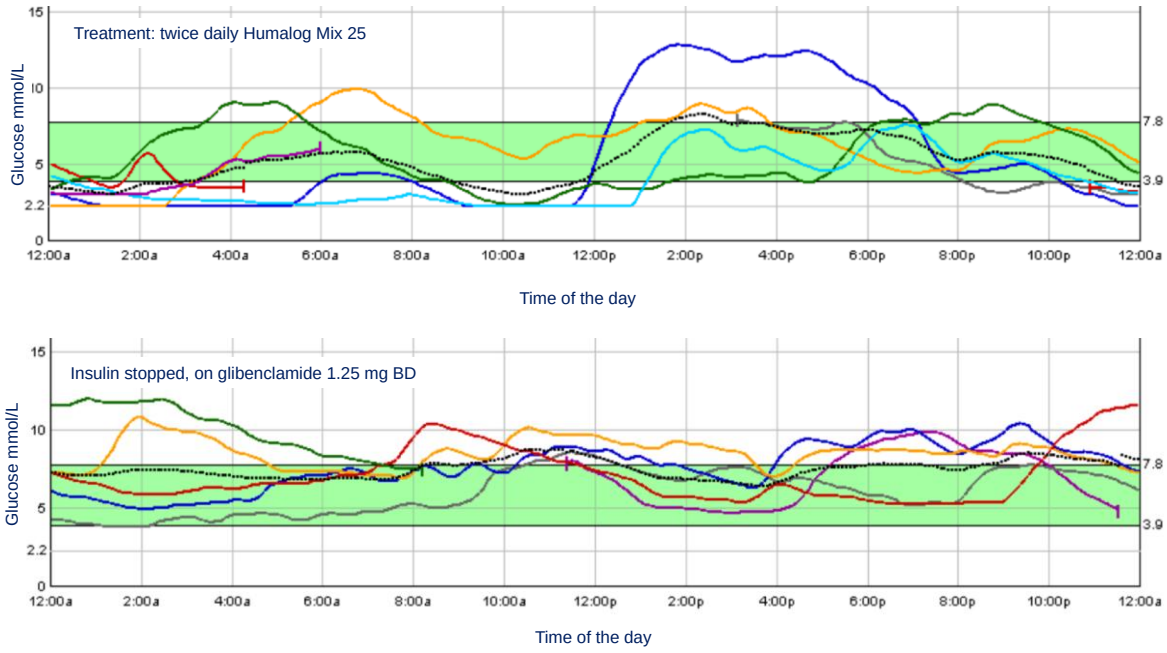
SUPPLEMENTARY DATA

Supplementary Figures

Supplementary figure 1. Family pedigree of proband and family members indicating genotype a diabetes status. Those affected with diabetes shaded black. Corresponding mutation status, if known. A251T = p.A251T variant detected and N = normal allele at same residue.



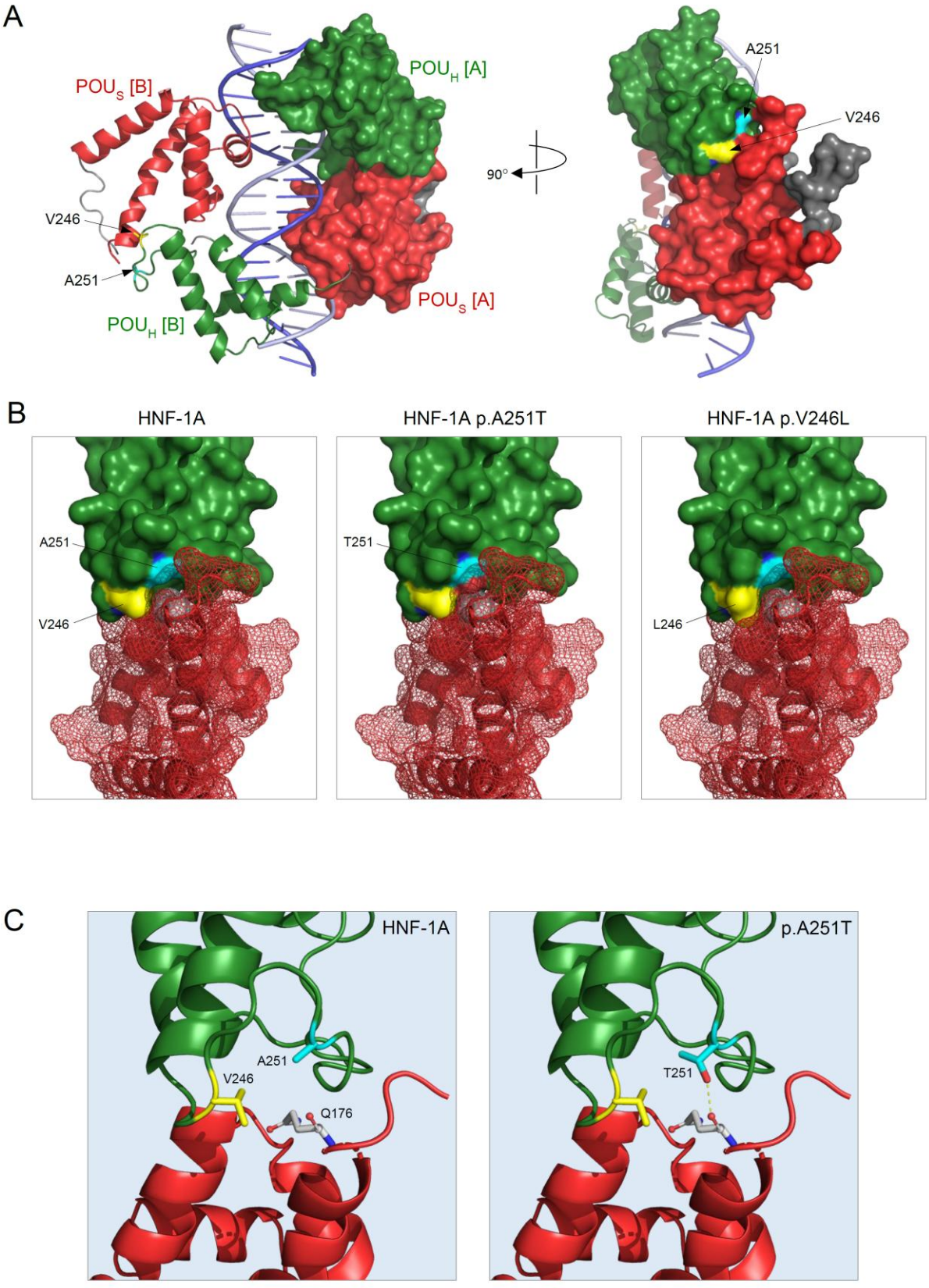
Supplementary figure 2. Retrospective continuous glucose monitoring using the iPro2 sensor (Medtronic, Northridge, CA), performed in the Proband before and after commencing sulphonylurea therapy. Upper panel, on twice daily pre-mixed insulin injections, lower panel on twice daily glibenclamide.



SUPPLEMENTARY DATA

Supplementary Figure 3. The p.A251T substitution affects the POU_H-POU_S interface in HNF-1A. A) Structure of HNF-1A residues 85-278 bound to DNA (PDB id 1ic8); HNF-1A binds DNA as a dimer, with one chain (1ic8A) shown in surface representation, and the other (1ic8B) in ribbon format; in both chains, the POU_S and POU_H sub-domains are coloured red or green respectively; residues A251 and V246 are indicated, with sidechain carbon atoms coloured cyan or yellow respectively. B) Rotated view of the POU_S-POU_H interface; the POU_S sub-domain is shown as a mesh surrounding the backbone ribbon; in both the p.A251T (centre) and p.V246L (right) variants, the bulkier sidechains of the novel amino acids are likely to impact adversely on close interaction with the POU_S sub-domain. C) In HNF-1A (left), the sidechains of both V246 and A251 lie within 6 Å of residue Q176 in the POU_S sub-domain; in the p.A251T variant (right), the novel threonine sidechain is predicted to form a direct hydrogen bond (yellow dashes) with the backbone oxygen atom of Q176.

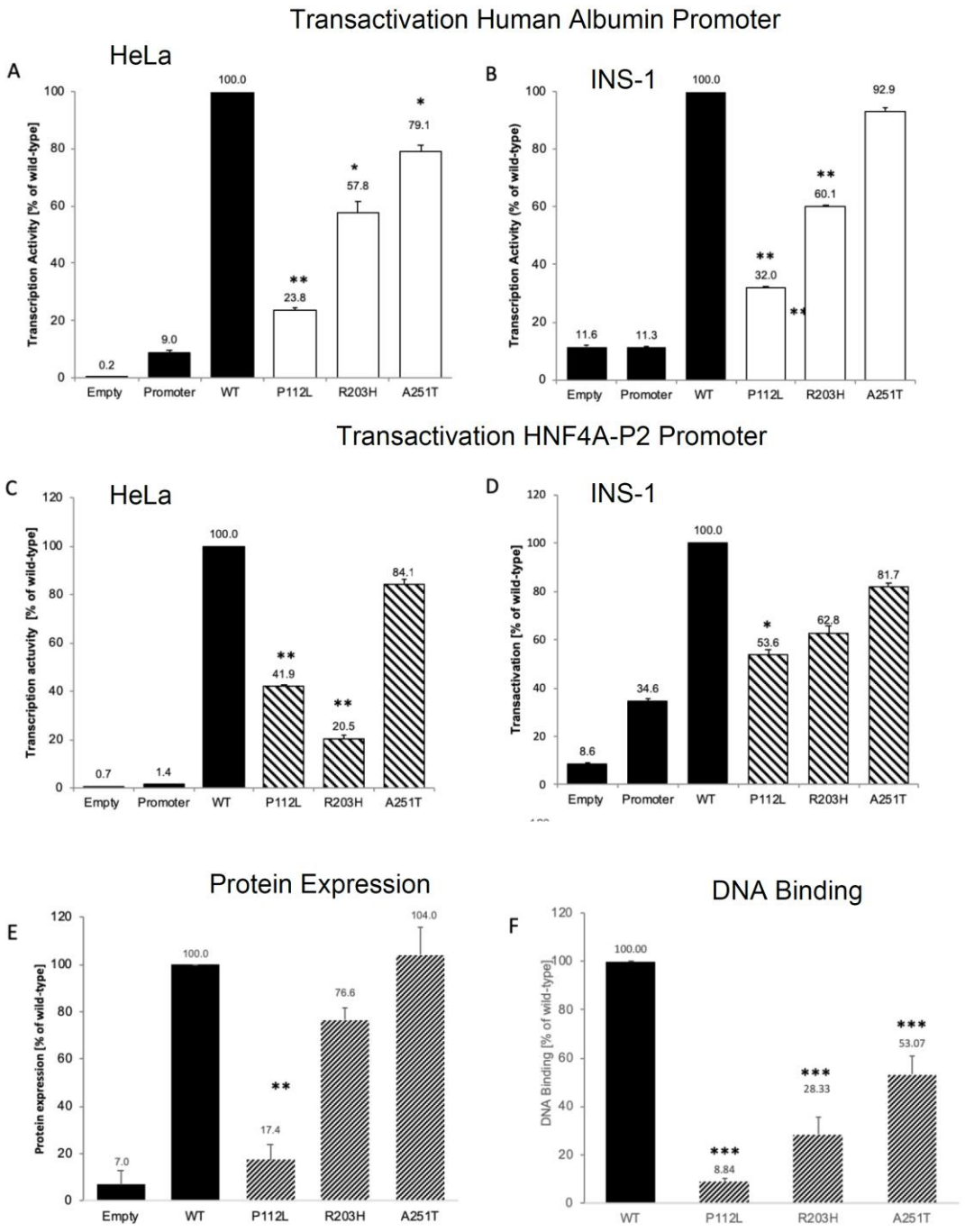
SUPPLEMENTARY DATA



SUPPLEMENTARY DATA

Supplementary Figure 4. Functional assessment of the p.A251T HNF1A variant**.** A: transcription activity using the luciferase reporter assay (n=4) in HeLA cells the human albumin promoter. B: transcription activity using the luciferase reporter assay (n=4) in INS-1 cells with the human albumin promoter. C: transcription activity using the luciferase reporter assay (n=3) in HeLA cells with the HNF4A-P2 promoter. D: transcription activity using the luciferase reporter assay (n=3) in INS-1 cells with the HNF4A-P2 promoter. E: Protein expression on Western blot analysis of HeLa cell-transfected protein lysates (n=3). F: DNA binding by electrophoretic mobility shift assay (EMSA). We used site-directed mutagenesis to introduce single nucleotide changes into *HNF1A* cDNA (NM_000545) and then cloned each construct into the pRK5 or pcDNA 3.1/HisC vector (ThermoFisher, Waltham, MA, USA). All data are presented as a mean percentage of the WT HNF1A with error bars. WT and negative controls in black, positive control variants in white, stripes or hatched. *p < 0.05, **p<0.01, ***p <0.001.

SUPPLEMENTARY DATA



SUPPLEMENTARY DATA

Supplementary Table 1. Characteristics of family members studied. A251T denotes the p.A251T allele; N denotes the normal allele; hsCRP, high-sensitivity C-reactive protein.

	HNF1A **Mutation Status**	**Age at diagnosis / study (years)**	**Duration (years)**	**BMI (kg/m^2)**	**Treatment at initial assessment**	**HbA1c % (mmol/mol)**	**Random C-peptide (pmol/L)**	**Random glucose mg/dL (mmol/L)**	**hsCRP mg/L**
Proband	A251T/A251T	15	13	27.8	Twice daily pre-mixed insulin	7.5 (58)	200	133 (7.4)	<0.2
Identical twin	A251T/A251T	12	16	28.7	Twice daily pre-mixed insulin	8.3 (67)	263	196 (10.9)	0.2
Sister (1)	A251T/A251T	16	19	23.2	Insulin glargine and insulin lispro (not taking)	12.2 (110)	155	126 (7)	<0.2*
Sister (2)	Not available for testing	18	unknown	unknown	unknown	unknown	unknown	unknown	unknown
Mother	A251T/N	29	34	25.4	Metformin & 10mg glibenclamide	8.7 (72)	502	248 (13.8)	0.2
Father	A251T/N	65	n/a	29.1	n/a	6.4 (46)	651	81 (4.5)	1.4

*An hsCRP of 0.8 mg/L was recorded at a subsequent assessment.

SUPPLEMENTARY DATA

Supplementary Table 2. Summary of findings from *in silico* testing to predict pathogenicity of the A251T variant. GnomAD, genome aggregation database; dbSNP, single nucleotide polymorphism database; SIFT, sorting intolerant from tolerant; PolyPhen2, polymorphism phenotyping version 2; $POU_{H/S}$, pituitary-octamer-unc DNA-binding (H) homeodomain and (S) specific, sub-domains.

Parameter	Description of finding
Mutation Database searches	Heterozygous p.A251T variant identified in a patient diagnosed aged 43 years, treated with Metformin and sulphonylurea for 25 years from diagnosis
Sequence Variant Database	Not listed in GnomAD, dbSNP, Exome Variant Server or 1000 genomes
Amino Acid Change	Alanine (non-polar) to Threonine (uncharged polar)
Species conservation	Conserved in 16 out of 17 orthologs, including 10 mammalian orthologs
In silico prediction	Likely pathogenic: SIFT, PolyPhen2 & Align-GVGD Uncertain: Grantham Distance is 58 (>50 pathogenic)
Cryptic Splice Site	Not predicted to create splice site
Protein structural modelling	Mild alteration of POU_H/ POU_S interface of the HNF1A DNA binding domain, but not as deleterious as confirmed mutations in the same area.